AD_____

Award Number: W81XWH-08-1-0220

TITLE:
Role of IRS1 and IRS2 in Modulating ErbB-induced Tumorigenesis

PRINCIPAL INVESTIGATOR:
Beate Litzenburger

CONTRACTING ORGANIZATION:
Baylor College of Medicine
Houston, TX, 77030

REPORT DATE:
May 2009

TYPE OF REPORT:
Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

√ Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-05-2009	Annual Summary	04-14-2008 – 04-13-2009

4. TITLE AND SUBTITLE
Role of IRS1 and IRS2 in Modulating ErbB-induced Tumorigenesis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-1-0220

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Beate Litzenburger

Email: litzenbu@bcm.tmc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Baylor College of Medicine, Houston, Texas, 77030

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and
Materiel Command
Fort Detrick,
Maryland, 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Insulin receptor substrates 1 and 2 (IRSs) are adapter proteins which are required for the transforming ability of many oncogenes. Up to date, little is known about the interaction between IRSs and ErbB2 in epidermal growth factor receptor (ErbB2/Her2/Neu) amplified breast cancer. In a mouse study we found that overexpression of IRS2 had no effect upon ErbB2 induced mammary tumorigenesis in vivo. Whole mounts and hematoxylin and eosin (H&E) analysis revealed no major morphological differences in ductal branching and tumor type(solid adenocarcinomas) between ErbB2 and bigenic (ErbB2/IRS2) overexpressing mice. Furthermore, the metastatic potential of ErbB2 is not changed by additional overexpression of IRS2.

15. SUBJECT TERMS
Insulin Receptor Substrate (IRS), IRS1, IRS2, epidermal growth factor receptor (EGFR/ErbB), ErbB2,

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	17	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

INTRODUCTION

Insulin receptor substrate 1 and 2 (IRS1 and IRS2) are adapter proteins that link signaling from upstream activators to multiple downstream effectors. IRSs modulate and coordinate multiple signaling cascades involved in normal growth, metabolism and survival suggesting that they may play a role in cancer. Indeed, IRSs are required for the transforming ability of many oncogenes and IRSs are elevated and hyperactive in many human tumors and high IRS1 levels are associated with poor prognosis in breast cancer. Adapter proteins have been shown to play an important role in epidermal growth factor receptor (ErbB2/Her2/Neu) amplified breast cancer. However, there is little known about the IRS interaction with ErbB2 in cancer development and progression. **Therefore, I hypothesize that ErbBs bind and phosphorylate IRSs, and that levels of IRSs will modulate ErbB-induced tumorigenesis.** The significance of this research project is the realization that IRSs are not simply mitogenic and metabolic signaling elements, but that they have numerous other functions that strongly implicate them in cancer development and progression. I believe that this proposal will increase our understanding of ErbBs in cancer development and progression, and may provide evidence and strategies for inhibiting IRSs as a therapeutic strategy in breast cancer.

BODY

1) Research and Training Accomplishments

The Breast Center at Baylor College of Medicine (BCM) provides a unique training environment with multiple opportunities for me to grow as a young research scientist. In the past year, I have taken full advantage of these opportunities and will outline my primary accomplishments here.

- attended and presented data in poster format at the Department of Medicine Research Symposia (April 2008)
- attended and presented data in poster format at the Gordon Conference on "Insulin-like growth factors in physiology and disease" (March 2009)
- audited a Translational Breast Cancer Research course taught here at BCM by faculty members of the Breast Center (December 2008)
- contributed a section to a text book chapter entitled "Insulin-Like Growth Factor Signaling in Normal Mammary Gland Development and Breast Cancer Progression," which was published in 2008
- contributed to a review article entitled "The Type-I Insulin-Like Growth Factor Receptor Pathway: A Key Player in Cancer Therapeutic Resistance"; this article largely focuses on the role of the insulin-like growth factor pathway in mediating resistance to numerous cancer therapies, such as radiation and chemotherapy, and targeted therapies, such as Tamoxifen and trastuzumab (May 2008)
- Published a first author research article entitled "BMS-536924 Reverses IGF-IR-Induced Transformation of Mammary Epithelial Cells and Causes Growth Inhibition and Polarization of MCF7 Cells". This article was published in Clinical Cancer Research describing a preclinical study suggesting that targeting IGF-IR may be an effective strategy for the treatment of human breast cancer (January 2009).

2) Research Project

IRS1 and IRS2 are adaptor proteins that link signaling from upstream activators to multiple downstream effectors to modulate normal growth, metabolism, survival, and differentiation. IRSs can interact with, and are functionally required for the transforming ability of many oncogenes, and are elevated and hyperactive in breast cancer. A recent protein microarray showed that IRS1 bind ErbB1 and ErbB2 [1]. We have shown that MCF10A cells overexpressing IRS1 or -2 are hypersensitive to EGF. In determining the mechanistic explanation for this, we found that EGF can phosphorylate and activate IRSs. **Therefore, I hypothesized that ErbBs bind and phosphorylate IRSs, and that levels of IRSs will modulate ErbB-induced tumorigenesis.**

During the first year of the project I have mainly focused on the role of IRS-2 in ErbB2-mediated tumorigenesis in transgenic mice. This was initially planned as Specific Aim 4, however the laboratory had set up the breeding before this award as these experiments can take a

long time. I will therefore first discuss the results from Specific Aim 4 as this has direct impact upon the other three specific aims.

Specific Aim 4: **Determine whether elevated (MMTV-IRSs) IRS levels affect ErbB2-induced tumorigenesis.**

As described in my proposal Aim 4 was designed to test the effect of cross-talk between ErbB2 and IRSs on mammary tumorigenesis in vivo. Therefore we set up the following mouse study: Homozygous MMTV-ErbB2 mice were bred with heterozygous MMTV-IRS2 mice to produce 2 cohorts for study – heterozygous MMTV-ErbB2 and bigenic heterozygous MMTV-ErbB2/MMTV-IRS2. The study was performed in multiparous (2 pregnancies) mice, since we have shown that IRS-induced tumorigenesis is significantly enhanced by pregnancy [2]. Multiparous MMTV-IRS2 transgenic female mice developed tumors significantly faster with a mean time to tumor formation (MTTF) of 68 weeks compared to virgin mice (MTTF = 95 weeks) with a p-value <0.001, probably due to the hormonal stimulation of MMTV transgene expression during pregnancy and lactation [2]. Female mice were housed 4 per cage and palpated twice a week. Animals were harvested when tumors reached 1000mm^3.

We first examined the effect of IRS-2 and ErbB2 overexpression on normal mammary ductal outgrowth in adult transgenic ErbB2 and bigenic ErbB2/IRS2 mice. Several studies support the importance of the GH/IGF-1 axis and respective receptors in mammary gland branching. IGF causes outgrowth of primary branches during development. In addition evidence also indicates that the transmembrane tyrosine kinase and potential EGFR partner ErbB2 influences ductal morphogenesis [3]. Finally, any effect on normal development would likely alter subsequent tumorigenesis thus we felt it important to first examine the normal mammary gland.

Primary branches as well as secondary side-branches were counted manually in 10 bigenic and 9 transgenic ErbB2 whole mounts which is summarized in table 1. Representative whole mounts show no difference in the ductal morphogenesis (Figure 1A). Bigenic mice developed 32% primary branches whereas ErbB2 transgenic mice developed 30% primary ducts which is not significantly different (p-value = 0.37) (Figure 1B). We didn't observe any significant difference in secondary side branching between the bigenic and ErbB2 mice with 68% and 70% secondary ductal outgrowth (p-value = 0.15), respectively (Figure 1B). Thus, we concluded that IRS2 does not alter branching morphogenesis in adult ErbB2 overexpressing mice.

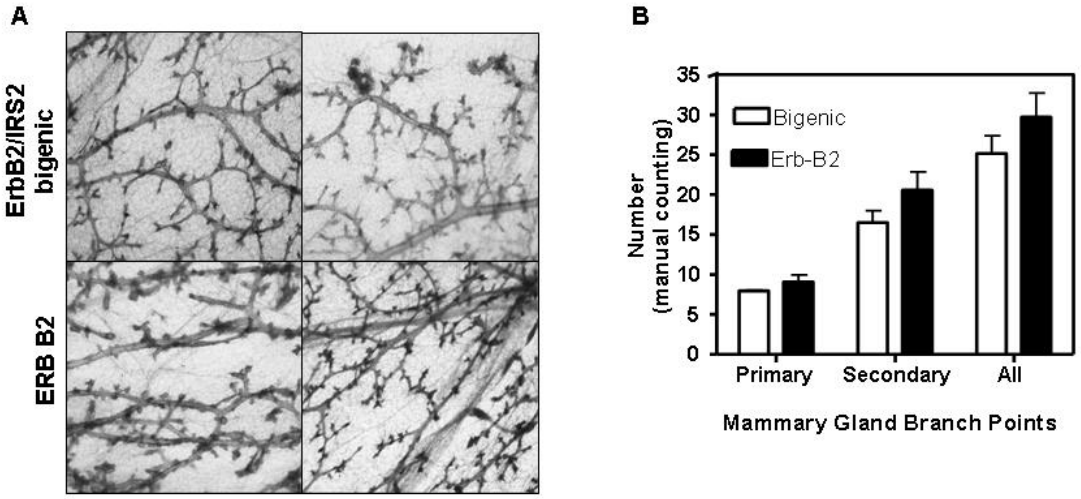

Figure 1: A. Representative whole mounts of mammary glands from adult ErbB2/IRS2 bigenic mice and ErbbB2 transgenic mice. Pictures were taken at 2x magnification. **B**. Quantification of manually counted mammary primary and secondary branch points.

Table 1: Summary of whole mount analysis and primary and secondary branch points

	Number of whole mounts	**Primary (%) 90˚**	**Secondary (%) 60˚**	**Total All**
Bigenic	10	7.94 ± 0.80 (32%)	16.5 ± 1.49 (68%)	25.15 ± 2.23
ErbB2	9	9.06 ± 0.88 (30%)	20.56 ± 2.27 (70%)	29.67 ± 3.04
P value		0.37	0.15	0.24

Given that there was no major difference in normal mammary gland development in ErrB2 vs. ErrB2/IRS-2 transgenic mice, we next compared the median time to tumor formation (MMTF) by Kaplan-Meier plots in the same sets of mice. Table 2 illustrates the percent of animals without mammary gland tumors versus the day tumors were first palpated. We found no significant difference in tumor development between ErbB2 transgenic (MTTF 30.5 weeks) and bigenic mice (MTTF 30.5 weeks). When tumors reached approximately 1000m^3, they were harvested and a representative part was cut for histological analysis. At the time of harvest we observed macro-metastasis in both groups.

Table 2: Comparing Groups: End of Tumor Study Summary

	MMTV-ErbB2	MMTV-ErbB2/ MMTV-IRS2
Number of Tumors	28 (31) **90%**	21 (24) **88%**
MTTF	30.5 wks/ 7.6 m	30.5 wks/ 7.6m
Lung Metastasis	Yes	Yes
Average number of tumors per animal	2.43	2.05

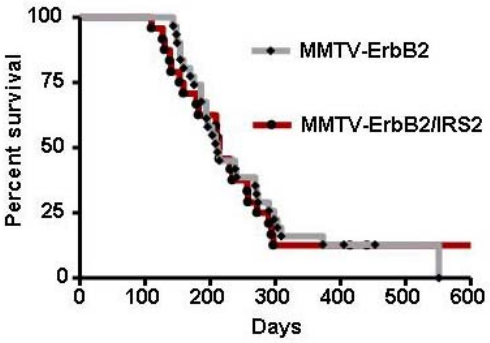

Figure 2: Kaplan-Meier tumor curve illustrates the percent of animals without mammary gland tumors versus the day tumors were first palpated comparing MMTV-ErbB2 transgenic mice to bigenic ErbB2/IRS2 mice. Bigenic mice initiated mammary tumorigenesis at the same rate as ErbB2 transgenic mice alone. Table 2: 90% of females with ErbB2 overexpression, and 88% of the bigenic mice developed tumors with a MMTF of 30.5 weeks or 7.6 months respectively.

We next examined the histology of ErbB2 overexpressing tumors and compared them with mammary tumors overexpressing ErbB2 and IRS2. Histological analysis was performed by H&E staining of 25 ErbB2 mammary tumors and 24 bigenic mammary tumors. ErBB2 tumors were predominantly adenocarcinoma (88%, 22 out of 25 tumors), which lack myoepithelium, keratinization or squamous metaplasia. Some of the ErbB2 tumors had dense stroma with lymphocytic infiltrates. Only two tumors display squamous carcinomas (8%, 2 out of 25 tumors) (Table 3).

In contrast to ErbB2 tumors, which are predominantly adenocarcinoma Dearth at al. reported that MMTV-IRS2 induced tumors exhibit only 20% solid nodular adenocarcinomas. Most of the IRS-2 transgenic tumors are highly differentiated characterized by ductal architectures or less-differentiated tumors without ductal structure. Highly-differentiated tumors display extensive squamous differentiation, dense stroma with lymphocytic infiltrates, keratinization, and/or glandular acini formation with lactating properties [2].

We found that 66% of the ErbB2/IRS2 bigenic tumors are predominantly undifferentiated solid nodular mammary tumor with sparse stroma being absent of myoepithelial cells and squamous metaplasia. Interestingly, bigenic tumors are more differentiated compared to ErbB2 transgenic mice alone. Differentiated tumors displayed squamous differentiation with 21% of all bigenic tumors being squamous carcinomas, other tumors showed characteristics of a papillary tumor with defined cords of branched ductal architecture or glandular acini formation with lactation (Table 3).

In conclusion, overexpression of IRS and ErbB2 resulted in tumors which are more differentiated than ErbB2 tumors. However, the difference in the tumor histology does not correlate with a faster tumor growth as shown previously in the Kaplan Meier survival plots.

Table 3: Histological characteristics of mammary tumors from ErbB2 transgenic and bigenic mice

Histological types	MMTV-ErbB2 25 tumors/18 animals	MMTV-ErbB2/MMTV-IRS2 24 tumors/17 animals
Undifferentiated		
Solid Adenocarinoma	88% (22/25)	66% (16/24)
Differentiated		
Adenosquamous Carcinoma	none	4% (1/24)
Squamous Carcinoma	8% (2/25)	21% (5/24)
Papillary	None	4% (1/24)
Myoepithelium	None	None
Stromal reaction	Some 28% (7/25)	Some 33% (8/24)
Inflammatory infiltrate	Necrosis	Necrosis
Lactation	4% (1/25)	4% (1/24)
Keratination	K14 positive (ER/PR negative) (no swirls)	K14 positive (ER/PR negative) (no swirls)
Angiogenesis	76% (19/25)	33% (8/25)

Table 3: H&E staining of mammary tumors promoted by ErbB2 or bigenic (ErbB2/IRS2) overexpression were analyzed for histology. Histological analysis showed that ErBB2 tumors were predominantly adenocarcinoma. In contrast bigenic tumors were more differentiated characterized by squamous carcinoma, papillary structures with defined cords of branched ductal architecture and lactation.

While characterizing th e hi stology we observed that Er bB2 tum ors exhibited higher angiogenesis com pared to bigenic tum ors (76% vs. 33%, Table 3). New blood vessel development is cr itical f or tum or growing and spreading and thus is an im portant process in tumor progression. Neovascularization influences the dissem ination of cancer cells to distinct sites and the vascularization level of a solid tum or is thought to be an ex cellent indicator of its metastatic p otential [4]. Thus we next com pared th e m etastatic po tential of M MTV-ErbB2 tumors and bigenic tumors by examining lungs from mice when tumors reached 1000mm^3.

Metastatic lung tum ors were observed in 50% of ErbB2 transgenic m ice (9 out of 18 lungs, Table 4), and 53% of ErbB2/IRS2 bigenic m ice (7 out of 17 lungs). The majority of the metastasis is found in blood vessels, which ar e considered as non i nvasive (Figure 3A). However, 7 out of 49 (14%) m icro-lung metastasis in the ErbB2 transgenic m ice and 10 out of 31 (32%) micro-lung metastasis in the bigenic group were invasive. These lung tumors presented characteristics of the prim ary tumor (Figure 3B). We observed that each metastatic lung had at least one a denocarcinoma, other m etastasis a re m ore dif ferentiated sim ilar to their p rimary tumors (Figure 3B).

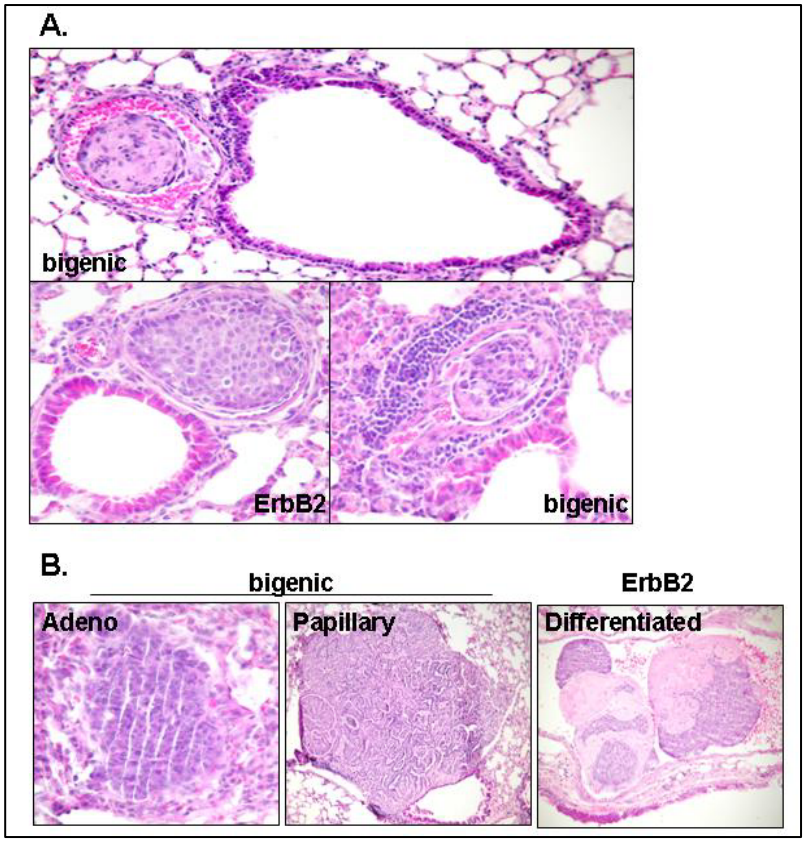

Figure 3: Representative H&E staining of lung metastasis promoted by ErbB2 and by bigenic (ErbB2/IRS2) overexpression. For detection of lung metastasis, sections were cut at intervals of 100µm through one half of the lung and all sections were stained by hematoxylin and eosin (H&E) and then examined microscopically. We scored lungs positive for lung metastases if they contained lesions with more than 100 cells. A. The majority of the metastasis of both study groups was found in blood vessels, which is considered as non invasive. B. H&E images of lung metastasis showing characteristics of the primary mammary tumor. All pictures were taken at 20x magnification.

In summary, we observed that ErbB2 tumors exhibited higher angiogenesis compared to bigenic tumors (76% vs. 33%, Table 3). However, this does not correlate with a higher metastatic potential of MMTV-ErbB2 with 50 % metastatic lung tumors compared to 53% in ErbB2/IRS2 bigenic mice.

Table 4: Histological characteristics of lung tumors from ErbB2 transgenic and bigenic mice

Animal	Lung Tumor	Number of metastasis/tumor (macro + micro)	Macro	Micro	Tumor Type
ErbB2	50% (9/18)	6.5 9 animals	6/9 mice 1.7 tumors per mouse	9/9 mice 5.4 tumors per mouse	• Adenocarcinoma • Differentiated lung tumor • Apoptotic
Bigenic	53% (7/17)	5.4 7 animals	3/9 mice 2 tumors per mouse	7/9 mice 4.4 tumors per mouse	• Adenocarcinoma • Papillary lung tumor • Differentiated lung tumors • Apoptotic

The data I presented to date showed that overexpression of IRS2 has no effect upon ErbB2 induced mammary tumorigenesis. Whole mounts and H&E analysis revealed no major morphological differences in ductal branching and tumor type (solid adenocarcinomas) between

ErbB2 and bigenic animals. Furthermore, the metastatic potential of ErbB2 is not changed by additional overexpression of IRS2.

This data directly contradicts my hypothesis and suggests that either there is no interaction between ErbB2 and IRS2 or that IRS2 is not required for modulation of ErbB2 action. It is not clear yet if there is truly no cross-talk between ErbB2 and IRS 2. To directly assess whether there is any functional interaction I will examine interaction between IRSs and ErbB2 (Specific Aims 1 and 2) and I will knockdown expression of IRS2 in ErbB2 mammary cancer cells in vitro. I expect that there maybe no association, or biological effect, of IRS-2 knockdown which would contradict my hypothesis and question the relevance of previous studies that suggest there maybe an important function of IRS2 downstream of ErbB2 in breast cancer.

Specific Aim 1: **Test whether IRSs bind ErbBs in vitro and in vivo, and if ErbBs directly phosphorylate IRSs:**

I have begun preliminary investigation into whether IRS2 co-immunoprecipitate with ErbB2 using immortalized MCF-10A human mammary epithelial cells that stably overexpress either HA-tagged IRS2 [2]. I was successfully able to pull down HA tagged IRS2. However co-immunoprecipitation showed little or no interaction between HA tagged IRS2 and ErbB2 or EGFR, respectively. My preliminary experiments are yet inconclusive. In my future experiments I will include a positive control, showing the interaction between IRS2 and IGF-IR.

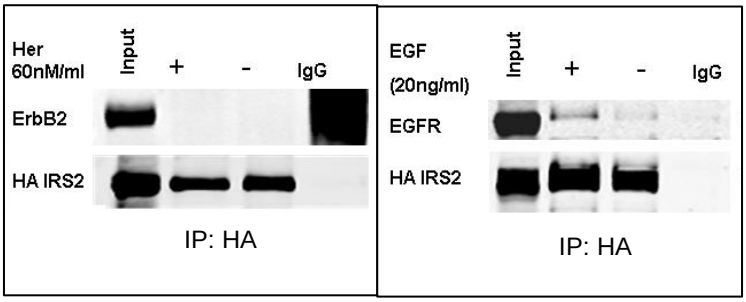

Figure 4: MCF10A cells stably expressing HA tagged IRS2 were starved in serum-free medium (SFM) overnight and then treated for 15 min with Heregulin (60ng/ml) or EGF (20ng/ml). IRS2 was immunoprecipitated with an HA antibody and protein levels were detected by Western blot using antibodies against either HA, ErbB2 or EGFR.

To more completely characterize the role of IRS2 in modulating ErbB2 based upon our results from Specific Aim 4 (and this was commented on in the peer review of my project), I plan to investigate the association between ErbB2 and IRS2 by co-immunoprecipitation in mouse mammary tumors cells from MMTV-ErbB2 transgenic mice. Dr. Brown kindly provided us mouse cell lines which were generated from MMTV-ErbB2 mice. I performed one initial experiment to determine the expression level of ErbB2 and also IRS2 in these cell lines. Immunoblot analysis confirmed ErbB2 overexpression and presence of IRS2 in all 3 mouse cell lines. I'm continuing to plan co-immunoprecipitation in the next couple of months. I hope to resolve the current issue as to whether there is an interaction between IRS2 and ErbB2.

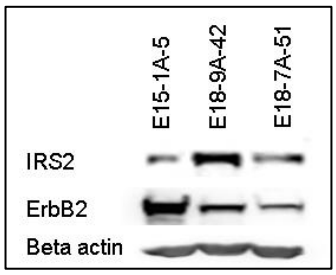

Figure 4: MMTV-ErbB2 mouse cell lines express ErbB2 and IRS2. The mouse cell lines E15-1A-5, E18-9A-42, E18-7a-51 were cultured in complete medium. Protein levels were detected by immunoblot analysis using antibodies against IRS2 and ErbB2 or beta actin.

All our previous observations during our mouse study lead us to the assumption that there is no requirement of IRS2 in ErbB2 action. To test this hypothesis, I will therefore perform transient reduction of IRS2 levels in the MMTV-ErbB2 mouse cells with siRNA and examine effects on proliferation. I hope that these initial experiments answer the phenotype seen in the above described mouse study.

***Specific Aim 2*: Compare and contrast IRS signaling activated by ErbB versus IGF-IR**

I have performed a preliminary experiment using reverse phase protein lysate arrays (RPPA) to examine EGF signaling compared to IGF signaling. For this pilot experiment, MCF-7 breast cancer cells were stimulated with EGF or IGF-I and then lysates were printed on glass slides and hybridized with over 100 antibodies. The experiment was successful and we noted interesting differences in these signaling pathways (data not shown). Thus this technique is now available to examine how IRSs alter ErbB2-induced signaling. However, given the lack of biological effect in the transgenic mice, we will not perform this time consuming and costly experiment until we have strong evidence that IRSs are modulating the biology of ErbB2 induced tumorigenesis.

***Specific Aim 3:* Test whether IRSs are required for the disruption of polarity by ErbBs**

This aim has not yet been started.

KEY RESEARCH ACCOMPLISHMENTS

- IRS2 does not alter branching morphogenesis in adult ErbB2 overexpressing mice.
- Overexpression of IRS2 had no affect upon ErbB2-induced mammary tumorigenesis, with median time to tumor formation of 30.5 weeks, respectively.
- Overexpression of both oncogenes, IRS and ErbB2, resulted in tumors which are more differentiated than ErbB2 tumors. However, the difference in the tumor histology does not correlate with a faster tumor growth as shown previously in the Kaplan Meier survival plots.
- In summary, we observed that ErbB2 tumors exhibited higher angiogenesis compared to bigenic tumors (76% vs. 33%, Table 3). However, this does not correlate with a higher metastatic potential of MMTV-ErbB2 with 50% metastatic lung tumors compared to 53% in ErbB2/IRS2 bigenic mice.

REPORTABLE OUTCOMES

- Casa, A., **Litzenburger, B.**, Dearth, R., and Lee A.V. Insulin-like growth factor signaling in normal mammary gland development and breast cancer progression. Breast Cancer: Prognosis, Treatment, and Prevention. 2nd Ed. Editor: Jorge R. Pasqualini. New York, NY, 2008. 303-321.
- Casa, A., Dearth, R.K., **Litzenburger, B.C.,** Lee, A.V., and Cui, X. The type I insulin-like growth factor receptor pathway: a key player in cancer therapeutic resistance. Front Biosci. 2008 May 1; 13: 3273-87. Review.
- **Litzenburger BC**, Kim HJ, Kuiatse I, Carboni, J. M., Attar, R. M., Gottardis, M. M., Fairchild, C. R., Lee, A. V. BMS-536924 reverses IGF-IR-induced transformation of mammary epithelial cells and causes growth inhibition and polarization of MCF7 cells. Clin Cancer Res 2009; 15(1): 226-37.

CONCLUSION

IRSs are adapter proteins which are required for the transforming ability of many oncogenes. In addition, IRSs are elevated and hyperactive in many human tumors. Adapter proteins have been shown to play an important role in ErbB2 (Her2/Neu) amplified breast cancer. However, there is little known about the IRS interaction with ErbB2 in cancer development and progression. A better understanding of the how IRS modulate ErbB2 induced mammary tumorigenesis may provide evidence and strategies for inhibiting IRS as therapeutic target in breast cancer. The research I have performed so far suggests that IRS2 is not required for modulating ErbB2 induced tumorigenesis *in vivo*. However, the experiments in the past year still leave many more questions open that need to be addressed. My immediate goal is to assess whether IRS2 is directly binding to ErbB2 via co-immunoprecipitation. Additionally, I want to see whether loss of IRS2 affects ErbB2 function in cell lines. If I find no effect here, this would be strong evidence that IRS2 doesn't signal downstream of ErB2. While this would directly contradict my hypothesis, this is an important observation that challenges the rationale for the study and would be reported so as to make other investigators aware of the lack of interaction.

Over the past year I have also attempted to expand my studies. Recently, my mentor developed and published on a unique transgenic model overexpressing constitutively active IGF-IR (CD8-IGF-IR) which showed disrupted mammary gland development and rapid appearance of mammary gland tumors [5]. Accumulating evidence indicates that crosstalk occurs between IGF-IR and ErbB2/Her2, and in particular that this crosstalk may mediate resistance to anti-HER2 therapy [6]. To better understand the mechanism of crosstalk in tumorigenesis between these two oncogenes, we interbred heterozygous MMTV-CD8-IGF-IR mice with heterozygous MMTV-ErbB2 mice to generate 4 different genotypes: MMTV-CD8-IGF-IR/MMTV-ErbB2 (bigenic), MMTV-CD8-IGF-IR only, MMTV-ErbB2 only, and FVB/N wild type (wt). The data, to date, is promising. Bigenic mice developed tumors significantly faster than either of the transgenic mice alone. Currently, we are analyzing the histology by H&E staining and by Immunohistochemistry. This mouse study may provide us with clues of how the two pathways, IGF-IR and ErbB2, may interact.

REFERENCES

1. Jones RB, Gordus A, Krall JA, MacBeath G: A quantitative protein interaction network for the ErbB receptors using protein microarrays. Nature 2006; 439(7073): 168-74.

2. Dearth RK, Cui X, Kim HJ, et al.: Mammary tumorigenesis and metastasis caused by overexpression of insulin receptor substrate 1 (IRS-1) or IRS-2. Mol Cell Biol 2006; 26(24): 9302-14.

3. Sternlicht MD: Key stages in mammary gland development: the cues that regulate ductal branching morphogenesis. Breast Cancer Res 2006; 8(1): 201.

4. Psaila B, Lyden D: The metastatic niche: adapting the foreign soil. Nat Rev Cancer 2009; 9(4): 285-293.

5. Carboni JM, Lee AV, Hadsell DL, et al.: Tumor development by transgenic expression of a constitutively active insulin-like growth factor I receptor. Cancer Res 2005; 65(9): 3781-7.

6. Nahta R, Yuan LX, Zhang B, Kobayashi R, Esteva FJ: Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. Cancer Res 2005; 65(23): 11118-28.

APPENDIX

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Beate Litzenburger	**Predoctoral Fellow**
eRA COMMONS USER NAME LITZENBU	

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
RWTH Aachen, Aachen, Germany	M.Sc.	2000-2006	Molecular and Cellular Biology
Baylor College of Medicine, Houston, TX	Ph.D. (in progress)	2006-now	Molecular Biology

RESEARCH AND PROFESSIONAL EXPERIENCE:

12/2006-present Baylor College of Medicine, Houston, TX USA
Breast Care Center, Predoctoral fellow
PhD Thesis: "The role of IRS1/2 in modulating ErbB-induced tumorigenesis"

10/2005-07/2006 Baylor College of Medicine, Houston, TX USA
Breast Care Center, Master's student
Master Thesis: Characterization of a small molecule inhibitor in the treatment of breast cancer
Collaboration with Bristol Myers Squibb

06/2005-09/2005 Grunenthal GmbH, Aachen Germany
Research & Development, Intern
Project: Planning and monitoring of a clinical phase IIb analgesic study

01/2004-06/2004 Baylor College of Medicine, Houston, TX USA
Department of Molecular and Cellular Biology, Intern
RNA-interference as a gene therapy strategy for the treatment of Epidermolysis Bullosa Simplex

2003/2004 Medical Center 'Blondelst.' Aachen Germany
Assistant Medical Technician
Analysis of human serum of HIV patients using Flow Cytometry

HONORS AND AWARDS

2007-2008	Fellowship of the German Academic Exchange Service
2007	1st place oral presentation award winner at the Breast Center Retreat, Baylor College of Medicine, Houston, TX
2006	1st place poster award winner at the Breast Center Retreat, Baylor College of Medicine, Houston, TX
2004	Research scholarship, Department of Molecular and Cellular Biology, Center for Molecular Cutaneous Research, Baylor College of Medicine, Houston, TX ▪ Development of gene therapy strategies for epidermolysis bullosa simplex.
1999-2000	President's Honor Roll. Butler County Community College

PUBLICATIONS

Litzenburger BC, Kim HJ, Kuiatse I, Carboni, J. M., Attar, R. M., Gottardis, M. M., Fairchild, C. R., Lee, A. V. BMS-536924 reverses IGF-IR-induced transformation of mammary epithelial cells and causes growth inhibition and polarization of MCF7 cells. Clin Cancer Res 2009; 15(1): 226-37

Casa, A., **Litzenburger, B.**, Dearth, R., and Lee A.V. Insulin-like growth factor signaling in normal mammary gland development and breast cancer progression. Breast Cancer: Prognosis, Treatment, and Prevention. 2nd Ed. Editor: Jorge R. Pasqualini. New York, NY, 2008. 303-321.

Casa AJ, Dearth RK, **Litzenburger BC**, Lee AV, Cui X. The type I insulin-like growth factor receptor pathway: a key player in cancer therapeutic resistance. Frontiers in Bioscience 13, 3273-3287, May 1, 2008

Kim HJ, **Litzenburger BC,** Cui X, Delgado DA, Grabiner BC, Lin X, Lewis MT, Gottardis MM, Wong TW, Attar RM, Carboni JM, Lee AV. Constitutively active type I insulin-like growth factor receptor causes transformation and xenograft growth of immortalized mammary epithelial cells and is accompanied by an epithelial-to-mesenchymal transition mediated by NF-kappaB and snail. Molecular and Cellular Biology, April 2007, p. 3165-3175, Vol. 27, No. 8

ORAL PRESENTATIONS

IGF-IR inhibitor BMS-536924 causes growth inhibition and polarization of MCF-7 breast cancer cells in 3D culture (3nd Annual Breast Center Retreat, 2007, 1st place oral presentation prize)

POSTER PRESENTATIONS

Litzenburger B.C., Michael J. Toneff, Robert K. Dear th, Hyun-Jung Ki m, Isere Kuiatse, Ora Britton, Yi Li, Adrian V. Lee. Constitu tive ac tivation of the insulin-like growth f actor rec eptor accelerates ErbB2-induced mammary tumorigenesis
(Gordon Research Conference, Insulin-like growth factors in physiology and disease, 2009)

Litzenburger B.C., Kim H.J., Carboni J, Fairchild C. R., Gottardis M.M., W ong T.W ., Attar R.M, Lee A.V. IGF-IR inhibitor BMS-536924 cau ses growth inhibition and polarization of MCF-7 breast cancer cells in 3D culture
(Department of Medicine Research Symposium, 2008)

Litzenburger B.C., Kim H.J., Carboni J, Gottardis M.M ., Wong T.W ., Attar R.M., Cui X., Lee A.V. Sm all m olecule inhibitor BMS-536924 completely reverses IGF-IR- mediated transformation of immortalized mammary epithelial cells
(Gordon Research Conference, Insulin-like growth factors in physiology and disease, 2007)

Litzenburger B.C., Kim H.J., Carboni J, Gottardis M.M ., Wong T.W ., Attar R.M., Cui X., Lee A.V. Sm all m olecule inhibitor BMS-536924 completely reverses IGF-IR- mediated transformation of immortalized mammary epithelial cells
(29th Annual San Antonio Breast Cancer Symposium, #4113, 2006)

Litzenburger B.C., Kim H.J., Carboni J, Gottardis M.M ., Wong T.W ., Attar R.M., Cui X., Lee A.V. Sm all m olecule inhibitor BMS-536924 completely reverses IGF-IR- mediated transformation of immortalized mammary epithelial cells
(NCI-AACR-EORTC, Prague, #564, 2006)

Litzenburger B.C., Kim H.J., Carboni J, Gottardis M.M ., Wong T.W ., Attar R.M., Cui X., Lee A.V. Sm all m olecule inhibitor BMS-536924 completely reverses IGF-IR- mediated transformation of immortalized mammary epithelial cells
(2nd Annual Breast Center Retreat, #11, 2006, 1st place poster prize)